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Abstract

Many human genetic disorders are caused by mutations in protein-coding regions of DNA. Taking protein structure into account has therefore provided key insight into the molecular mechanisms underlying human genetic disease. While most studies have focused on the intramolecular effects of mutations, the critical role of the assembly of proteins into complexes is being increasingly recognized. Here we review multiple ways in which consideration of protein complexes can help us to understand and explain the effects of pathogenic mutations. First, we discuss disorders caused by mutations that perturb intersubunit interactions in homomeric and heteromeric complexes. Second, we address how protein complex assembly can facilitate a dominant-negative mechanism, whereby mutated subunits can disrupt the activity of wild-type protein. Third, we show how mutations that change protein expression levels can can lead to damaging stoichiometric imbalances. Finally, we review how mutations affecting different subunits of the same heteromeric complex often cause similar diseases, while mutations in different interfaces of the same subunit can cause distinct phenotypes.

Introduction

Alterations in the coding regions of the genome often affect gene function by changing the structure of their protein products. There are a number of possible biophysical effects of a single amino acid change in a polypeptide. These include an altered free energy landscape for the folding of the polypeptide, changed chemistry of an active site, or perturbed hydrogen- and ionic-bonding networks leading to a change in the general stability and dynamics of the folded structure. One important aspect that should not be ignored is that the crowded environment of the cell contains proteins that are in constant interaction with each other as well as with other macromolecules and metabolites. These interactions mean that proteins continuously take part in short-lived events such as those involved in signaling networks, as well as the formation of protein complexes and filaments that are stable over longer time scales. In most cases, a protein not only has to be able to fold into a specific structure, but must also be able to make specific interactions with other biomolecules in order to carry out its function. Thus, understanding these interactions and how they are affected by mutations will be crucial to understanding genetic disease. Indeed, Zuckerkandl and Pauling stated in 1962 that: “Life is a relationship between molecules, not a property of any one molecule. So is therefore disease”[1].

The development of next-generation sequencing techniques has been changing the perspective for diagnosis and personalized treatment of genetic disease. Large research coalitions are rapidly identifying new disease-related genes through widespread exome and genome sequencing[2]. The translation of genetic data into a deeper understanding of the mechanisms underlying disease is, however, far from straightforward. Even when the genetic basis of a disease is well understood, the specific molecular mechanisms that lead to the disease phenotype might not be. The correlation between a variant in the genome and the observed symptoms is often unclear, even for monogenic Mendelian disorders[3].

In this review we will take a protein structural view of disease-associated mutations and focus on those proteins that need to assemble into stable complexes in order to carry out their biological functions. Specifically, we will discuss several different ways in which thinking about protein complexes can aid our understanding of genetic disease. We will cover three different molecular mechanisms unique to complexes by which mutations can cause disease: by disrupting interactions, by inducing an assembly-mediated dominant-negative effect, and by causing stoichiometric imbalances. We will also review how knowledge of protein complex composition and structure can help to explain the phenotypic effects of different mutations.

Mutations that perturb protein interactions

Many if not most pathogenic mutations cause disease by inducing a simple loss of function at the protein level. In particular, it has been stated that most disease-associated missense mutations destabilize the structures of proteins and stop them from correctly folding, which is reflected in their strong enrichment at residues in the buried interiors of proteins[4–8]. Pathogenic mutations also often cause disease by affecting protein interactions: past work has consistently highlighted the tendency for them to be enriched at the interfaces of protein complexes and/or perturb protein interactions[9–14].

Protein complexes can be broadly divided into two categories. Homomeric complexes are formed from the assembly of multiple copies of the same polypeptide subunit. The formation of homomeric complexes is extremely common, with most known protein complex structures being homomeric[15] (although this is strongly influenced by the tendency of structural biologists to study individual proteins[16]). In contrast, heteromeric protein complexes are formed from at least two different polypeptide subunits, usually encoded by different genes. In this section, we will discuss examples of pathogenic human mutations that affect intersubunit interactions in both homomeric and heteromeric protein complexes.

Tubulin genes encode members of the tubulin protein family, of which the most common members, α- and β-tubulin, assemble into an obligate heterodimer that further polymerizes to form microtubules. Microtubules provide tracks for motor proteins, such as dynein and kinesins, to allow directional transport and a cytoskeletal scaffold with microtubule-associated proteins to build specific subcellular structures, including axons and the mitotic spindle. Pathogenic missense mutations, associated primarily with brain malformations, have been identified in β-tubulin genes, including TUBB4A, TUBB2A, TUBB3, α-tubulin gene TUBA1A and γ-tubulin gene TUBG1. Structural analysis of tubulin heterodimers reveals that these mutations are enriched at heteromeric interfaces within the microtubule complex. For example, the Asp249Asn mutation of the β-tubulin TUBB4A is found in approximately half of all reported cases of hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC)[17]. This highly conserved residue lies in the T7 loop, which is known to directly interact with GTP-bound α-tubulin (Figure 1). Given the severity of H-ABC, it is hypothesized that this mutation prevents the association of α/β tubulin heterodimers, thus preventing microtubule assembly[17]. Similarly, the Asn329Ser mutation in the α-tubulin TUBA1A is associated with severe lissencephaly with cerebellar hypoplasia. This residue is located at the α/β tubulin interface and stabilizes the interaction between H10 of α-tubulin and the B5-H5 loop of β-tubulin (Figure 1). It has been proposed that the mutation weakens the longitudinal interaction between tubulin subunits within a protofilament by disrupting proper hydrogen bonding across the interface[18]. Recent reports have revealed new pathogenic tubulin missense mutations, several of which occur at interfaces, including in TUBB8 causing female infertility by preventing correct meiotic spindle formation[19] and in TUBA4 and TUBB1 associated with blood disorders such as low platelet counts[20–23]. We predict more isotype-specific tubulin missense mutations will be reported in other disorders, given the central role of tubulin in all eukaryotes.

Deficiency in the homotetrameric medium-chain acetyl-CoA dehydrogenase (AD) enzyme, encoded by the ACADM gene, is a relatively common metabolic disorder[24]. Affected individuals have metabolic phenotypes such as problems with fasting, impaired ketogenesis and episodes of hypoglycemic coma, and prolonged deficiency can be fatal. The majority of medium-chain AD deficiency is caused by a Lys304Glu substitution at the homomeric interface, present in 90% of individuals with the condition. An alteration from the positively charged Lys to the negatively charged Glu acid disrupts ionic bonding as well as possible water bridges and affects the assembly of a functioning tetramer[25–27] (Figure 2A).

The mannose-binding lectin (MBL) protein plays an important role in innate immunity by attaching to foreign pathogens and activating the complement system via the lectin pathway[28]. Its function appears to be pattern recognition and it binds to carbohydrate patterns on the surface of a large variety of pathogens. MBL deficiency is a relatively common genetic condition that leads to recurring infections in affected people, of varying severity depending on the genetic variant. The MBL2 gene encodes MBL subunits that form a basic collagen-like triple helix unit. These subunits then oligomerize further into higher-order homomers, including pentamers and hexamers, which are complementary to distinct pathogen carbohydrate patterns. All the mutations that have been associated with MBL deficiency (Arg52Cys, Gly54Asp and Gly57Glu) are located in the collagen-like region of the protein and disrupt the initial assembly of the coiled-coil motif[29].

While the above examples represent highly penetrant alleles associated with monogenic Mendelian disorders, mutations that disrupt protein interactions can potentially have more subtle effects as well. ALDH2 encodes the homotetrameric mitochondrial aldehyde dehydrogenase complex. The Glu504Lys variant, which has an allele frequency of ~25% in East Asian populations, but is rare in others, has been associated with sensitivity to alcohol consumption[30] and susceptibility to alcohol-related cancers[31]. Interestingly, the substitution occurs at the core of the intersubunit interface (Figure 2B), which reduces its enzymatic

activity[32], and also weakens the interaction and thus reduces the stability of the complex[33]. There is also a dominant-negative aspect to this mutation: the heteromeric complex formed with mixed wild-type and mutant subunits is largely inactive, and the presence of mutant subunit also causes the wild-type subunit to be degraded at a much faster rate[33,34]. This phenomenon will be discussed in more detail in the following section.

The dominant-negative effect

An important mechanism by which mutations in protein complexes can cause disease is the so-called “dominant-negative effect”[35]. This has often been observed in proteins that assemble into symmetric homomeric or heteromeric complexes that contain multiple copies of the same type of subunit. If a mutation occurs in one allele of a protein-coding gene, then the complex will assemble as a mixture of wild type and mutated subunits. A dominant-negative effect occurs if the mutated subunit can block the function of the wild-type subunits, thus causing a disproportionate loss of function[36]. The greater the number of repeated subunits in a complex, the greater the potential for a dominant-negative effect (Figure 3). Although the dominant-negative effect is very well known to geneticists, there has been relatively little attempt to study it from a structural perspective. It is important to note that, although protein complex assembly appears to account for the large majority of known dominant-negative mutations, there are other potential molecular mechanisms by which a dominant-negative effect can occur[37]. In this review, our discussion is focused on the assembly-mediated dominant-negative effect.

Given that the dominant-negative effect is dependent on the mutant protein binding to the wild-type protein, we can hypothesize that dominant-negative mutations should be significantly milder at a protein structural level than other pathogenic mutations. If a mutation is highly disruptive to protein folding or blocks the protein interaction, this would generally not be compatible with a dominant-negative effect, as the mutant protein would not be able to

assemble into a complex. We recently performed an analysis of transmembrane channel mutations where we found that dominant-negative mutations tend to be much milder than other pathogenic mutations at a structural level, consistent with the idea that the dominant-negative effect requires mutant subunits to at least be stable enough to assemble into complexes[38]. This strongly suggests that consideration of protein complex structure could improve the prediction and identification of dominant-negative mutations.

It has been proposed that some tubulin mutations act in a dominant-negative manner, whereby mutant subunits can be incorporated into microtubules. For example, multiple mutations in TUBB3 have been associated with congenital fibrosis of extraocular muscles (CFEOM)[39]. Of these, Arg262His mutants were able to form heterodimers and permit subsequent incorporation of mutant heterodimers into microtubules. Conversely, Arg262Cys mutants demonstrated diminished heterodimer formation and microtubule incorporation. Notable Arg262His mutants are also associated with more a severe TUBB3 phenotype than Arg262Cys, which strongly supports a dominant-negative mechanism whereby increased mutant incorporation leads to a more severe phenotype[39]. TUBB4A demonstrates another example of the dominant-negative effect: Asn414Lys is associated with severe H-ABC and shows heterodimer incorporation at a level similar to wild type. In contrast, Val255Ile, which does occur directly in the heterodimeric interface (Figure 1) and is likely to perturb the interaction, presents with milder, isolated hypomyelination and a reduction in the amount of mutant heterodimer incorporation, consistent with a weaker dominant-negative effect[40]. TUBB3 Arg262 and TUBB4A Asn414 are both located on the outer surface of the microtubule (Figure 1) and substitutions at these positions are likely to interfere with how microtubule motors and microtubule-associated proteins associate with the microtubule lattice, thus explaining the more severe phenotypes associated with the dominant-negative mutations that get incorporated into microtubules.

Dominant-negative mutations in microtubule motor proteins have also been reported. The missense mutations prevent motor inhibition so that the microtubules become constitutively active; these gain-of-function mutations have thus sometimes been referred to as “dominant positive”. For example, missense mutations in the dynein adaptor protein BICD2 cause spinal muscular atrophy[41–43]. BICD2 is thought to be autoinhibited through intramolecular coiled coils, which can be alleviated by cargos such as the small GTPase Rab6[44,45]. Several pathogenic missense mutations (Ser107Leu, Asn188Thr, Ile189Phe and Phe739Ile) show an increased ability to assemble into motile dynein-dynactin-BICD2 complexes when stimulated by a cargo Rab6. As a result, the dynein complexes become hyperactive, enhancing retrograde transport in neurons and creating an imbalance in neuronal intracellular transport and changes in neurite length[46]. Similarly, missense mutations in KIF21A are associated with CFEOM[47]. The mutations cause loss of motor auto-inhibition and gain of function by disrupting the intramolecular interaction between the motor domain and the third coiled coil. The mutant motors associate with microtubules at a greater frequency than wild-type KIF21A, but their speed and run length is unaffected[48]. The ocular motor neurons expressing mutant KIF21A have changes in the neuronal morphology, in particular in the growth cone. Ocular motor neurons therefore seem very sensitive to the regulation of their neuronal microtubules, as shown by the motor and tubulin associated pathogenesis.

The signal transducer and activator of transcription (STAT) family of proteins are transcription factors that are activated by the binding of signaling proteins to cell-surface receptors, which triggers phosphorylation and subsequent homo- or heterodimerization. The activated STAT dimer is then transported to the nucleus where it drives gene expression[49]. Due to the dimeric nature of active STAT proteins, dominant-negative effects have been observed in a number of patients carrying certain STAT mutations. Minegishi *et al.* studied a cohort of hyper-IgE syndrome (HIES) patients that carried five distinct STAT3 mutations located in the DNA-binding domain of the protein[50]. They noted that patients carrying these

mutations exhibited levels of STAT3 protein comparable to that found in controls, with phosphorylation and dimerization capability unchanged from wild type. However, a dominant-negative effect was detected when measuring the DNA binding ability of STAT3 mutants that were co-expressed with wild-type STAT3. Compared to wild-type dimers, the DNA-binding ability of mutant STAT3 was severely compromised. This explained why these HIES patients displayed an impaired response to cytokine stimulation, despite phosphorylation and dimerization of STAT3 proceeding as normal following cytokine-receptor binding. Any STAT3 dimer containing at least one mutant monomer could no longer bind DNA and modulate gene expression. Interestingly, another study has shown how dominant HIES mutations located in a different region, the SH2 domain, exhibit impaired STAT3 phosphorylation, indicating that there may be more than one molecular mechanism that can lead to the same observed HIES phenotype[51]. As of yet, no null alleles have been discovered, which suggests that HIES is not primarily a result of STAT3 haploinsufficiency[52]. This indicates that these potentially disparate molecular mechanisms that result from mutations in different domains may all involve STAT3 dimers that display dominant-negative activity.

Mutation-induced imbalances in subunit stoichiometry

If the expression level of a heteromeric subunit is increased or decreased, this will cause an imbalance in the stoichiometry of the complex and can potentially be detrimental[53–55] (Figure 4). Such imbalances can occur due to heterozygous mutations in individual protein coding genes that either decrease or knockout the expression of the allele, or from larger scale copy number variations (CNVs) or aneuploidy[56,57]. There is considerable evidence supporting the idea that stoichiometric imbalances can be damaging, often referred to as the ‘balance hypothesis’. For example, evolutionary evidence points to small-scale subunit gene duplications that disrupt complex stoichiometry being unlikely, whereas genes retained after

whole genome duplications are disproportionately enriched in dosage sensitive subunits – presumably to maintain the stoichiometric balance[53,56,57]. Moreover, dosage compensation mechanisms may also exist in the form of post-translational degradation[58–62] or other forms of non-linear component buffering[63,64].

The need for finely-tuned stoichiometric balancing mechanisms is exemplified by the α/β tubulin dimer, and has been studied extensively in yeast. Tubulin expression has been shown to be auto-regulated post-transcriptionally through mRNA destabilization, which points to an evolutionary adaptation to conserve tubulin levels[65,66]. Despite this layer of regulation, tubulin stoichiometry perturbations manifest in deleterious phenotypes. Even modest levels of excess β-tubulin destabilize microtubules through promiscuous interactions, while strong overproduction is lethal[67]. Overproduction of a β-tubulin isoform has also been shown to have deleterious effects on microtubule stability in mammalian systems[68]. Conversely, excess α-tubulin is not detrimental, as it is subject to dosage compensation through degradation, and in fact rescues the β-tubulin toxicity phenotype when expressed at or above equal levels[69,70]. Additionally, Plp1p and Rbl2p – factors important in β-tubulin folding – were found to prevent toxicity from excess β-tubulin by forming dimers and eventually leading to β-tubulin aggregates[71,72]. These structures do not result in further toxicity, but instead were suggested to form through a mechanism intended to avoid off-target undimerized β-tubulin interactions. Finally, a recent discovery also demonstrates that tubulin heterodimers are stoichiometrically matched with tau proteins, the imbalance of which leads to formation of tau aggregates and neuronal dysfunction[73]. This multitude of systems that arose through the course of evolution to tightly control the relative stoichiometry in microtubule formation highlights the importance of expression balance in protein complexes.

A number of human disorders involving protein complexes have been suggested to occur due to subunit haploinsufficiency. For example, around 70% of heterozygous human

mutations in BMPR2, the type II receptor for bone morphogenetic proteins, were found to result in the introduction of premature termination codons that cause transcript nonsense-mediated decay[74]. This leads to patients developing pulmonary arterial hypertension as a result of aberrant signaling due to insufficient abundance of a receptor complex – a suggested consequence of subunit stoichiometric imbalances. Notably, abundance changes of COL5A1, the pro-α1(V) component of type V collagen, and COL2A1, the pro-alpha1(II) chain of type II, have been suggested to cause non-linear phenotypic effects due to stoichiometric imbalances in their specific protein complexes. Haploinsufficiency of these proteins has been previously shown to result in human diseases – causing Ehlers-Danlos syndrome and Stickler syndrome, respectively[75,76].

In spite of specific examples, global analyses of gene dosage sensitivity and balance in yeast have produced conflicting evidence in regards to the phenotype enrichment and conditional haploinsufficiency due to under- and over-expression or protein complex subunits[77–80]. More specifically, several yeast studies did not find that protein complex subunits are enriched in overexpression phenotypes, excluding a subset of structural proteins that include β-tubulin[77,80]. In addition, the different biases of overexpression techniques employed in these studies were shown to result in opposing enrichment of protein types[81].

There are a number of possible reasons why complex-related deleterious overexpression phenotypes remain elusive in populations. CNVs of protein complex subunits may not be observed in populations if they are severely deleterious and result in lethality, as exemplified by β-tubulin overexpression or moderate α-tubulin reduction under most backgrounds[67,69,82]. Alternatively, non-linear effects have been theorized to buffer under- and overexpression of subunits in a complex, depending on individual topological and assembly specifics, resulting in a disproportionate complex abundance, which is milder than expected[63,64]. Such buffering mechanisms have been postulated to be driven by an evolutionary optimization of subunit

dissociation levels[63]. Finally, some overexpressed proteins may be subject to dosage compensation, as illustrated by α-tubulin, and may avoid reaching deleterious levels[69]. Indeed, recent findings show that a majority of protein complex subunits are subject to dosage compensation mechanisms, which occur post-translationally and maintain component stoichiometric balance[58–62]. Assembly into complexes has been shown to stabilize subunits, while the majority of excess components are degraded. The ERAD and ubiquitin-proteasome pathways have been implicated in these processes, targeting mainly highly connected uncomplexed protein subunits, while peripheral components are likely to not be under compensation[58–60]. Additionally, studies have shown peripheral component CNVs to be more likely, and their knockouts or duplications not having significant effects[56,59]. This illustrates the evolutionary importance of conserving the stoichiometry of core subunits, while avoiding to overburden the dosage control machinery with functionally non-significant subunits that do not exhibit deleterious effects in excess. However, it is apparent that compensation mechanisms may not be able to cope with the excess subunits introduced by CNVs, exemplified by the lack of variation in the majority of complex subunits, and its main purpose may be to buffer against expression noise from internal and external perturbations[56]. In the future, more data from healthy populations may reveal if post-translational compensation is capable of obfuscating CNVs of complex components.

Subunit composition and quaternary structure can often explain mutation phenotype

Mutations in proteins that are part of a complex or are connected within an interaction network have a strong tendency to have similar phenotypic effects[83,84]. There are many examples of mutations in genes encoding different subunits of the same heteromeric complex can cause similar human genetic disorders. Thus, knowledge of the subunit

composition of protein complexes can potentially lead to better diagnoses of genetic disease.

Cohesin is a large complex with a crucial regulatory involvement in chromatid separation during cell division[85]. It is formed of a donut-shaped trimer containing a pair of elongated structural maintenance of chromosome proteins, SMC1 and SMC3, and a kleisin family protein RAD21. Numerous accessory proteins associate with these core subunits, including NIPBL from the Hawk family of condensin and cohesin regulators[86]. Cohesin forms a large ring structure that entraps sister chromatids during cell-division[87]. Mutations in all four genes have been associated with Cornelia de Lange (CdL) syndrome. Most people with CdL have slow growth and distinctive facial features, such as low set ears and long eyelashes, but the severity of the phenotype can vary highly even within patients with the same mutation[88]. Gene expression in Drosophila and mouse models of cohesinopathies is altered[89,90], suggesting cohesin mutations may affect transcription and genome-wide organization. Cells from CdL patients display an increased sensitivity to DNA damage and changes in gene expression[91,92]. While the disease mechanism remains unresolved, it is clear that perturbing cohesin function by mutating any of the core subunits can lead to similar phenotypes.

Aicardi-Goutieres Syndrome is associated with a large number of mutations in different genes, and is characterized by effects to the brain, such as progressive microcephaly and spasticity[93]. Most pathogenic mutations are in the RNASEH2A, RNASEH2B and RNASEH2C genes that encode the three subunits of the heterotrimeric type II ribonuclease H enzyme[94]. The molecular mechanism underlying the pathogenic mutations is not yet known, but the key component is a dysfunctional ribonuclease. As the inheritance pattern is autosomal recessive, we can speculate that the observed variants are likely to be highly destabilizing to the subunits themselves or the resulting complex. Reduced activity in the enzyme leads to a build-up of endogenous nucleic acids that triggers an inappropriate immune response without the presence of any viral pathogens[95]. Thus, damaging mutations in any of the

complex subunits will have similar loss-of-function effects and lead to similar disease phenotypes.

Branched-chain α-ketoacid dehydrogenase complex in the mitochondrial inner membrane is a very large enzyme complex crucial for the citric acid cycle[96]. The complex consists of three catalytic components, E1, E2 and E3, where E1 catalyses the carboxylation of the ketoacid. E1 is itself a hetero-tetramer made up of two α and two β subunits encoded by BCKDHA and BCKDHB. Variants of these two genes are related to maple syrup urine disease (MSUD), where the body is incapable of the breakdown of the branched amino acids, Ile, Leu and Val, which accumulate[97]. The name stems from the characteristic sweet odour from the urine of the infants, and the phenotype involves developmental delay and general lack of energy caused by rapid degeneration of brain cells. MSUD type 1a is caused by mutations in the E1-α subunit, the most common of which (Gly290Arg, Phe409Cys and Tyr438Asn) are all located directly at the intersubunit interface and therefore interrupt the formation of a functioning complex[97]. Mutations in the β subunits lead to MSUD type 1b, with an overlapping phenotype to type 1a. Arg183Pro allele is very common in MSUD-carriers and occurs β-sheet close to the interface with one of the α subunits. The incorporation of the proline residue in the β-sheet hydrogen bond network causes dramatic structural rearrangements that disrupt the interaction between the α and β subunits[98].

Despite heteromeric protein complex formation being somewhat predictive of disease phenotype, as the examples above illustrate, there are cases where different mutations in the same complex cause distinct, clinically separated phenotypes[99]. When analyzing the missense mutation pairs on the same gene, Wang et al. found that mutations affecting different interaction interfaces are more than twice as likely to cause different distinct disorders[84]. There was no such difference indicated in between mutation pairs on domains affecting non-interacting proteins, which further highlights the importance of taking protein interactions in to account.

An example of mutations at different interfaces having different phenotypes is the optineurin protein encoded by the OPTN gene. Known missense mutations associated with associated with familial amyotrophic lateral sclerosis (ALS) all occur at homodimeric interfaces (Arg96Leu, Gln454Glu, Met468Arg, Glu478Gly)[100–102], although they do occur in different domains involved in homodimerization (Figure 5). In contrast, missense mutations associated with glaucoma occur at heteromeric interfaces formed with TBK1 (Glu50Lys)[103,104] or polyubiquitin (His486Arg)[105], or on the protein surface (Met98Lys)[106]. Thus it is likely that the ALS phenotype is due to disrupted dimerization, while glaucoma may be due to impaired interactions with other proteins.

Conclusions

As we have shown here, a complex-centric view of protein mutations is crucial for understanding the molecular mechanisms underlying many human genetic disorders. While proteomic approaches have revealed the subunit compositions of many complexes, the three-dimensional structure of a complex is often needed to interpret the mechanisms discussed here. Thus, a major limitation in our understanding of human genetic disease comes from the fact that the structural coverage of the human proteome and interactome is still far from complete. In particular, heteromeric complexes have often been more difficult to study using high-resolution structural biology, due to their often large sizes, the difficulty of obtaining sufficient homogenous complexes, and the limitations of co-expression and recombinant systems that make them unsuitable for X-ray crystallography and NMR. However, in recent years, our ability to structurally characterize heteromers has grown dramatically due to tremendously improved co-expression systems, purification of complexes from native sources and advances in cryo-electron microscopy, which has revolutionized the structural characterisation of large heteromeric complexes[107].

Another critical issue comes from the fact that, of those tools that have been developed to use protein structural information to predict the effects of mutations, many only consider the structures of monomeric subunits, ignoring intermolecular contacts within the complex. Furthermore, even among those tools that do consider protein complexes, some have been built to work with the PDB asymmetric unit, which may or may not correspond to the biologically relevant quaternary structure. Such tools should always be programmed to use the PDB biological assemblies, or be flexible in terms of input quaternary structure.

Finally, it important to emphasize that there are other mechanisms by which protein complexes can be important for genetic disease beyond those discussed here. For example, although we discussed mutations that are disruptive to protein interactions, there are likely to be damaging mutations that have their effect by increasing the strength of an interaction or modulating binding specificity. In addition, protein complexes, particularly homomers, are often associated with allosteric behaviour, and this is likely to have significant implications for predicting the effects of mutations[108]. There is also the phenomenon of mutation-induced protein aggregation and the formation of toxic amyloids[109]. Finally, considerable attention has been paid in recent years to the phenomenon of phase separation mediated by many cooperative weak protein interactions, which also may be modulated by mutations[110].

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Figures

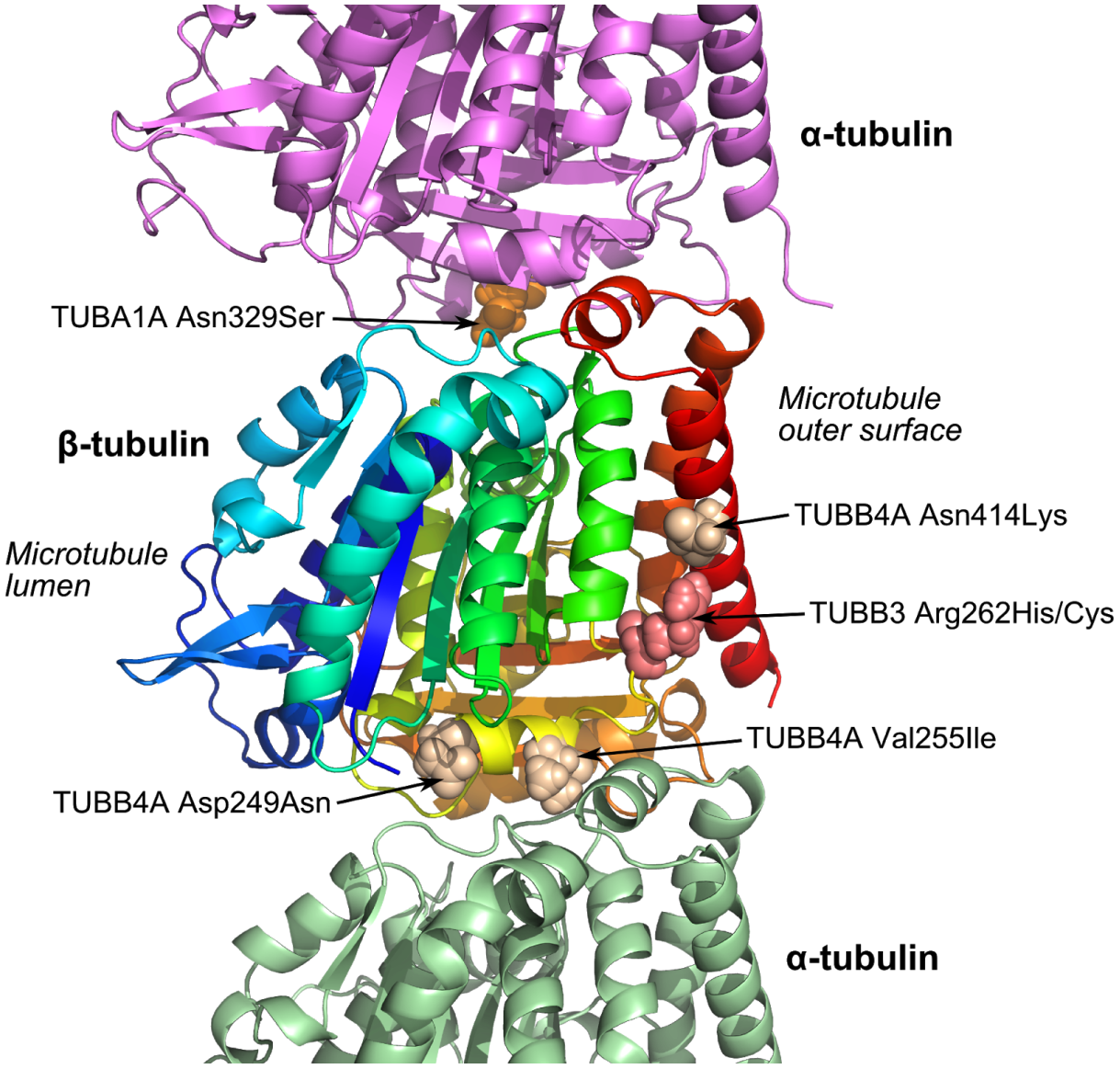

Figure 1: Structural context of pathogenic tubulin mutations. The sites of several mutations discussed in the text are highlighted on the structure of an α/β tubulin complex (PDB ID: 4I4T). β-tubulin is coloured as a rainbow from the N-terminus (blue) to the C-terminus (red).

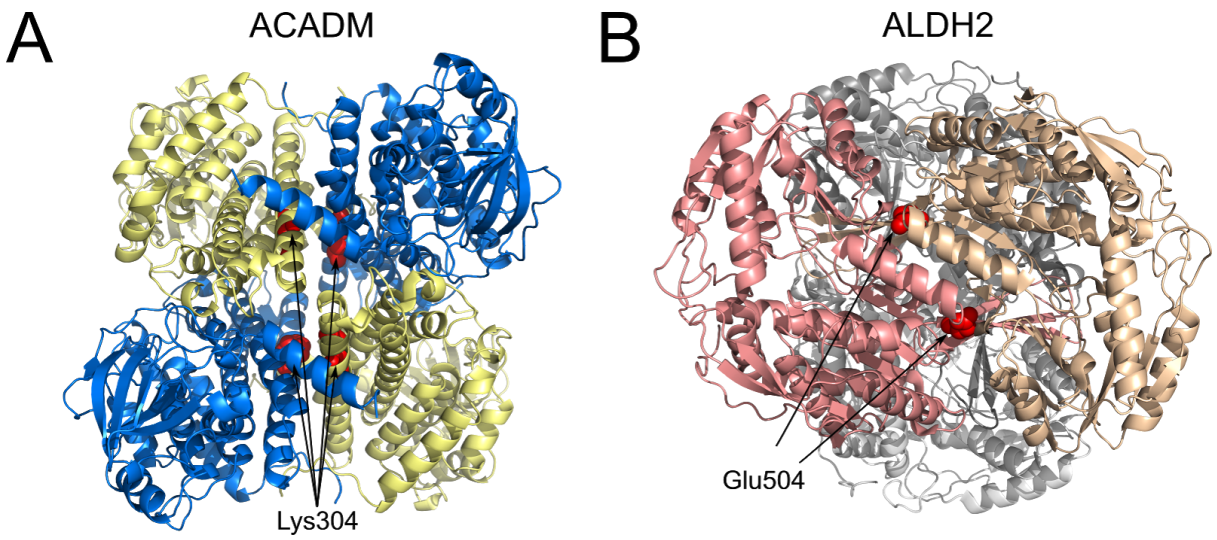

Figure 2: Damaging mutations at homomeric interfaces. (A) Structure of medium-chain acetyl-CoA dehydrogenase, encoded by the ACADM gene (PDB ID: 1EGE). (B) Structure of mitochondrial aldehyde dehydrogenase complex (PDB ID: 3N80).

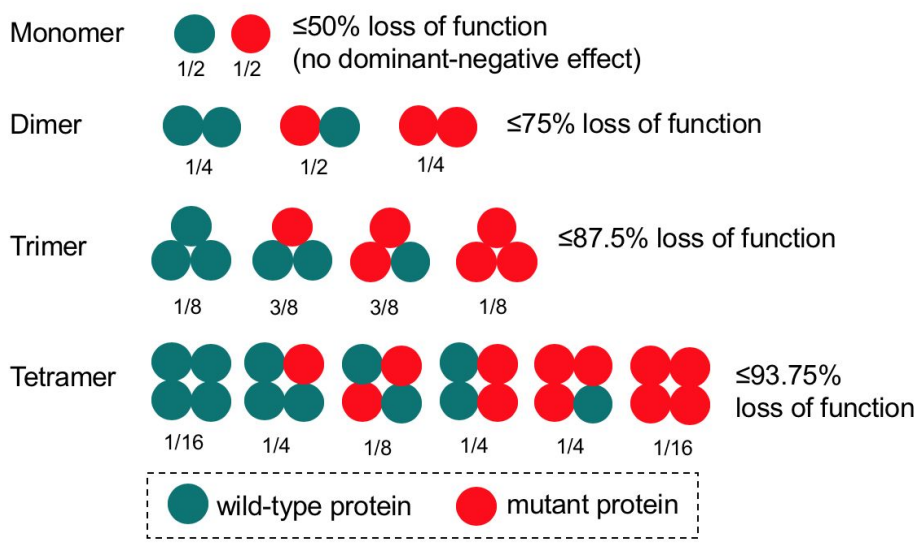

Figure 3: Illustration of the dominant-negative effect in homomeric complexes. For a monomer encoded by a heterozygous allele, half of the proteins will be wild type and half will be mutant; therefore, at most the mutation can cause a 50% loss of function. If the protein forms a homodimer, then 3/4 dimers will contain at least one mutant subunit; thus, if the presence of a mutant subunit blocks the activity of the entire complex, this could cause a disproportionate 75% loss of function. As the number of repeated subunits in the homomer increases, the proportion of complexes containing at least one mutant subunit and the potential for a dominant-negative effect also increase.

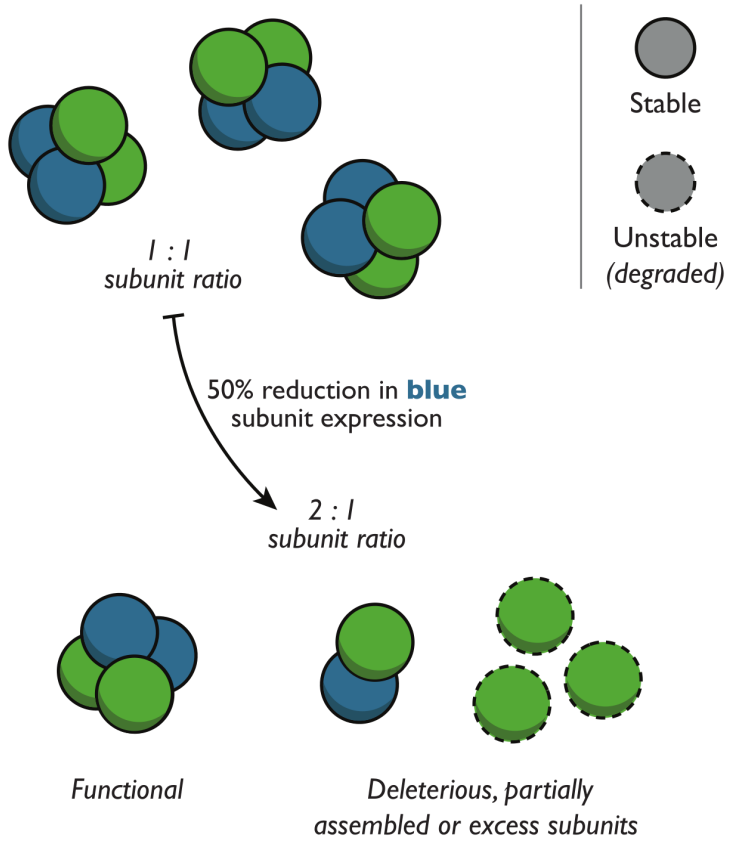

Figure 4: Stoichiometric imbalance in a heteromeric complex. Under normal conditions, the green and blue subunits that make up the 2:2 heterotetramer are expressed at equal levels. However, if there is a heterozygous knockout mutation that results in a 50% reduction in expression of the blue protein, there will now be a stoichiometric imbalance, with two green subunits being produced for each blue subunit. Thus, there will be fewer functional heterotetramers formed, and there will be an excess of green protein that can potentially have damaging effects itself.

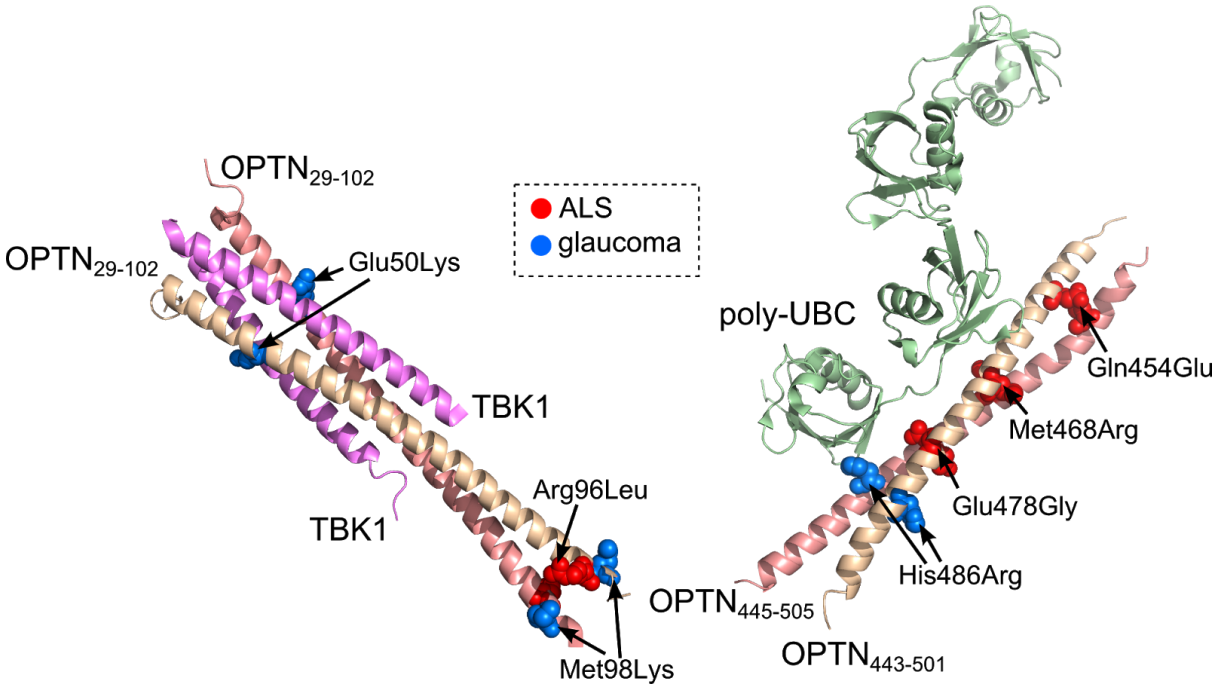

Figure 5: Pathogenic mutations in optineurin. Sites of mutations associated with ALS are shown in red, while sites of mutations associated with glaucoma are shown in blue. Two complexes are shown: one representing an N-terminal region of optineurin (residues 29-102) in complex with TBK1 (PDB ID: 5EOF), and one representing a C-terminal region of optineurin in complex with polyubiquitin (PDB ID: 5B83).